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Evaluation of food and nutrient intake assessment using concentration biomarkers in European adolescents from the Healthy Lifestyle in Europe by Nutrition in Adolescence study

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Abstract

Accurate food and nutrient intake assessment is essential for investigating diet–disease relationships. In the present study, food and nutrient intake assessment among European adolescents using 24 h recalls (mean of two recalls) and a FFQ (separately and the combination of both) were evaluated using concentration biomarkers. Biomarkers included were vitamin C, β-carotene, DHA + EPA, vitamin B_{12} (cobalamin and holo-transcobalamin) and folate (erythrocyte folate and plasma folate). For the evaluation of the food intake assessment 390 adolescents were included, while 697 were included for the nutrient intake assessment evaluation. Spearman rank and Pearson correlations, and validity coefficients, which are correlations between intake estimated and habitual true intake, were calculated. Correlations were higher between frequency of food consumption (from the FFQ) and concentration biomarkers than between mean food

Abbreviations: DIAT, Dietary Assessment Tool; FA, fatty acid; HELENA, Healthy Lifestyle in Europe by Nutrition in Adolescence.

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intake (from the recalls) and concentration biomarkers, especially for DHA + EPA (*r* 0·35 *v.* *r* 0·27). Most correlations were higher among girls than boys. For boys, the highest validity coefficients were found for frequency of fruit consumption (0·88) and for DHA + EPA biomarker (0·71). In girls, the highest validity coefficients were found for fruit consumption frequency (0·76), vegetable consumption frequency (0·74), mean fruit intake (0·90) and DHA + EPA biomarker (0·69). After exclusion of underreporters, correlations slightly improved. Correlations between usual food intakes, adjusted for food consumption frequency, and concentration biomarkers were higher than correlations between mean food intakes and concentration biomarkers. In conclusion, two non-consecutive 24 h recalls in combination with a FFQ seem to be appropriate to rank subjects according to their usual food intake.

Key words: **European adolescents: Food intake: Nutrient intake: Biomarkers: Validation studies: Triads method**

Accurate assessment of food intakes of free-living persons and especially of children and adolescents remains a difficult and labour-intensive process. Precise estimations are essential, however, especially with regard to the investigation of diet–disease relationships[1]. Problems in the accurate estimation of individuals' usual intake levels, together with the relative homogeneity of food consumption patterns within populations make it difficult to accurately estimate the disease risk associated with specific dietary factors[2–4]. Measurement errors can be divided into random errors and bias or systematic errors. Random errors may be a function of day-to-day variations in intake or errors in the analysis of food composition. Random errors in the classification of subjects according to their usual intakes can bias risk estimates and reduce the likelihood of detecting a significant association between diet and disease. Systematic errors in dietary data can inflate or deflate the relative risk or OR, depending on the direction of the bias and whether the source of the bias in dietary intake data is related to the disease outcome variable[1].

No single assessment method of an individual's usual intake is optimal under all conditions. The choice of method depends on a number of factors including the aim of the study, the characteristics of the study population, the accuracy of the dietary data required, and the funds and personnel available[5,6]. Repeated 24 h recalls have been shown to be a valid method to measure protein, K, fish, vegetable and fruit intakes among adults[7,8], though little is known about the validity among children and adolescents. Also, FFQ have been widely used as cost-effective dietary assessment methods in large-scale surveys to investigate usual food intakes. Both dietary intake assessment methods (repeated 24 h recalls and FFQ) have been used in the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) study[9]. Like all dietary assessment methods, estimates derived from 24 h recalls and FFQ data suffer from random and systematic errors and may not represent the 'true' usual intake of foods and nutrients. In general, repeated 24 h recalls have less bias and a larger within-individual variation than FFQ and vice versa[5,6].

The advantages of nutritional biomarkers have been previously shown[10]. For example, the random errors occurring with their utilisation are likely to be independent of those in both 24 h recall and FFQ[6]. It is likely that there is some degree of correlation between random errors in 24 h recall and FFQ, as both methods rely on the subject's ability of recalling and describing food consumption[10]. Also, errors due to underreporting could occur when using 24 h recalls or FFQ[11–13]. The inclusion of biomarkers in dietary validation studies makes it more likely that the criteria of independent errors are met[14].

The use of concentration biomarkers in validation studies is restricted to their associations with self-reported dietary intakes because these biomarkers are the result of complex metabolic processes[15]. Evaluated concentration biomarkers include serum carotenoids[16–19] and serum vitamin C[19,20] for fruit and vegetable intake and *n*-3 fatty acids (FA) for fish and seafood intake[21–23].

Strong correlations of dietary intakes of vitamin C and serum ascorbic acid concentrations have been reported mainly when habitual dietary intakes of vitamin C are relatively modest[24]. As many factors influence serum folate concentrations and the bioavailability of dietary folate, intakes may correlate only weakly with serum concentrations unless broad categories of folate intake are used[25]. Erythrocyte folate concentrations correlate with liver folate levels and thus reflect folate stores[26]. Total serum vitamin B_{12} concentration reflects both the vitamin B_{12} intake and body stores. Weak but positive correlations were reported for males and females between dietary vitamin B_{12} intake and serum vitamin B_{12}[27]. Low correlations may be linked to the large size of liver vitamin B_{12} stores. However, holo-transcobalamin is the only circulating transport protein that delivers vitamin B_{12} to receptors on cell membranes and is the only biologically active form of the vitamin[6]. Reported correlations between FA in serum phospholipids and FA intake vary markedly across studies[28,29] due to the fact that many factors may influence measured FA biomarkers in serum.

The purpose of the present study was to evaluate food and nutrient intake assessment among European adolescents from two 24 h recalls and an FFQ using concentration biomarkers. Fruit and vegetable intake *v.* vitamin C status and β-carotene status and fish intake *v.* the sum of DHA and EPA status were included to evaluate food intake. In addition, vitamin B_{12} (cobalamin and holo-transcobalamin), folate (plasma folate and erythrocyte folate), vitamin C, β-carotene, and the FA DHA and EPA were used to evaluate nutrient intake.

Subjects and methods

The HELENA Cross-Sectional Study is a multi-centre investigation of the nutritional and lifestyle status of adolescents in ten European cities[30].

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures

involving human subjects/patients were approved by the Human Research Review Committee of the Universities of Bonn (Dortmund), Lille, Rome, Zaragoza, Athens, Heraklion, Pécs, Ghent and Vienna. Informed written consent was obtained from participants and both parents.

Subjects, recruitment and study design

For the purpose of this study, ten cities of more than 100 000 inhabitants located in nine European countries were included: Vienna (Austria), Gent (Belgium), Lille (France), Dortmund (Germany), Athens and Heraklion (Greece), Pécs (Hungary), Rome (Italy), Zaragoza (Spain) and Stockholm (Sweden)[30]. A random cluster sampling of 3000 European adolescents (target number) aged 12·5–17·5 years, stratified for geographical location, age and socio-economic status, was carried out. Adolescents were recruited from school sites.

Up to three classes from two grades were selected per school and a class was considered eligible if the participation rate in the class was at least 70 %. A random subgroup of approximately 1000 adolescents (target number) was selected to participate in the blood sampling. Adolescents were excluded from participation *a posteriori* if they were participating simultaneously in another clinical trial, if they were aged $<12{\cdot}5$ or $\geq 17{\cdot}5$ years if they had suffered from an acute infection less than 1 week before the inclusion.

All data were collected via standardised procedures. Details on the sampling and recruitment process and quality-control procedures can be found elsewhere[31].

Ethical issues and respect for good clinical procedures have also been discussed previously[32].

Dietary assessment

Dietary intake was assessed using a computerised 24 h recall on two non-consecutive days of the week, excluding weekend days, and within a time-span of 2 weeks. In addition, a FFQ was completed by the adolescents. On the day of the first 24 h recall, a blood sample was taken.

The 24 h recall was assessed using a validated computer-based tool for self-reported 24 h recalls, the HELENA-Dietary Assessment Tool (DIAT), based on a previous version developed for Flemish adolescents, called Young Adolescents' Nutrition Assessment on Computer[33,34]. This HELENA-DIAT guides respondents through six 'meal occasions', embedded within questions that help the respondents to remember what they ate the day before. For each meal occasion, adolescents were invited to select all food items eaten at that occasion from a standardised menu. For each selected item, one or more extra screens were provided to gather detailed information on portion sizes. Additional features of the program are: probing for food items often eaten in combination with other items, a search engine, an extra category to add unavailable items, a number of checks (e.g. for extreme amounts, zero values, beverages, energy of consumed food, milk when cereals are consumed) and the possibility to add a remark before leaving the program.

Difficulties in obtaining comparable measures of kJ/g across countries precluded the use of country-specific food composition tables to calculate energy and nutrient intakes. Specifically, the national food composition tables were often limited in the number of foods that were included or in the number of nutrients to be analysed. Additionally, the definition of some nutrients differed between tables. To address this, the data of the HELENA-DIAT were linked to the German Food Code and Nutrient Data Base (BLS (Bundeslebensmittelschlüssel), version II.3.1, 2005)[35], as this food composition database contained the largest number of nutrients and food items: approximately 12 000 coded foods, menus and menu components with up to 158 nutrient data points available for each product. Data from each country were linked to this database to ensure standardisation of available measures. If a food item was missing in the German food composition table, calculations were made via recipes or a local food composition table for the specific country.

Furthermore, a short self-administered FFQ with fifteen items was used from the Healthy Behaviour in School-aged Children study[36], including fruits, vegetables, sweets, soft drinks, light soft drinks, cereals, white bread, brown bread, skimmed milk, whole-fat milk, other milk, cheese, fish, crisps and French fries. Response categories included: never, less than once a week, once a week, 2–4 times a week, 5–6 times a week, once a day every day and more than once a day every day.

The database architecture and easy-to-enter, user-friendly software for data input were centrally developed in Teleform and MSAccess. The questions from the multiple response formats were scanned centrally, whereas the open entry data were entered and archived by each local field centre.

Analysis of biomarkers in blood

After a 10 h overnight fast, blood from the antecubital vein was drawn between 08.30 and 10.00 hours at school following a standardised blood collection protocol. Details about the transport of the samples, quality assurance and stability pilot study can be found elsewhere[37]. Briefly, for the measurement of plasma folate, cobalamin and vitamin C, blood was collected in heparinised tubes, immediately placed on ice, and centrifuged within 30 min (3500 ***g*** for 15 min). Vitamin C samples were stabilised with metaphosphoric acid[37].

The supernatant fluid was transported at a stable temperature of 4–7°C to the central laboratory at the University of Bonn (IEL, Germany) and stored there at −80°C until it was assayed. Serum samples for FA and β-carotene analysis were clotted at room temperature for at least 30 min and then centrifuged (3500 rpm, for 15 min). Aliquots for FA analysis were stored locally at −20/−80°C as soon as possible. Once all fieldwork of one study centre was completed, all FA samples were shipped on dry ice to IEL and stored at −80°C until analysis. After measuring the haematocrit *in situ*, EDTA whole blood was sent to IEL for the erythrocyte folate analysis. EDTA whole blood was diluted 1:5 with freshly prepared 0.1% ascorbic acid for cell lysis, and incubated for 60 min in the dark before storage at −80°C. Erythrocyte folate was measured by competitive immunoassay (Immulite 2000; DPC

Biermann GmbH). Sera for measuring holo-transcobalamin were obtained by centrifuging blood collected in evacuated tubes without anticoagulant at 3500 ***g*** for 15 min within 1 h. Once sent to IEL, the sera were aliquoted and stored at −80°C until transport on dry ice to the biochemical laboratory at the Universidad Politécnica de Madrid for analysis.

Analyses of vitamin C, β-carotene, cobalamin, plasma folate, erythrocyte folate, DHA and EPA were performed centrally at IEL. Cobalamin and plasma folate were measured in heparin plasma by means of a competitive immunoassay using the Immunolite 2000 analyser (DPC Biermann GmbH). Antioxidative nutrients (β-carotene and vitamin C) were analysed by HPLC (Sykam Gilching) using UV detection (UV-Vis 205, Merck). Serum FA concentration was determined by capillary GC (Model 3900, Varian GmbH) after extraction performed by TLC. FA were identified by comparison of the peaks of interest with the retention times of authentic fatty acid methyl esters (FAME) standards (Sigma-Aldrich). The absolute FA profile was expressed as μmol/l. The relative amount of each FA (%) was expressed as the percentage of total concentration. The relative amount of DHA + EPA was expressed as the percentage of total FA concentration (% DHA + EPA). Holotranscobalamin was measured at Universidad Politécnica de Madrid by microparticle enzyme immunoassay (Active B_{12} Axis-Shield Limited) with the use of AxSym (Abbott Diagnostics, S.A.).

Statistical analyses

The software package STATA 10.1 was used (StataCorp). Only adolescents with complete biomarker, 24 h recall and FFQ data were included in the evaluation of food intake assessment. For the evaluation of nutrient intake assessment, only adolescents who provided a blood sample and who performed a 24 h recall twice were included. Data from the FFQ were recalculated to consumption frequency per d (continuous variable). The mean food and nutrient intakes from both recall days were used in the analyses. Descriptive analyses of the study population were performed.

For food intake assessment evaluation, unadjusted Spearman rank correlations were computed between concentration biomarker and mean food intake, concentration biomarker and frequency of food consumption, and mean food intake and frequency of food consumption. Unadjusted Spearman rank correlations to study the relationship between food intake assessment and biomarker were calculated for fruit intake *v.* vitamin C status, vegetable intake *v.* vitamin C status, fruit intake *v.* β-carotene status, vegetable intake *v.* β-carotene status and fish intake *v.* sum of DHA and EPA status. Pearson's correlation coefficients (after logarithmic or square root transformation) were included as sensitivity analysis.

Furthermore, the triads method was used to evaluate the correlation between the three measurements (FFQ, biomarker and 24 h recall) and the true intake using validity coefficients[14,38,39]. The triads method is a triangular comparison between questionnaire, biomarker and 24 h recall used to obtain a quantitative estimate of validity coefficients[14]. The technique assumes linearity between the three measurements and the true intake and assumes that the measurements have independent random errors. The assumption of independence implies that the correlations between any pair of variables are due to the relationship between each variable and the actual intake and not due to errors inherent in each assessment instrument (FFQ, 24 h recalls and biological markers)[14,39,40]. Pearson correlations were used to calculate the validity coefficients. The advantage of this method is the inclusion of the biomarker, which presents independent errors compared to the other dietary assessment methods, FFQ and 24 h recalls. Limitations of this technique include the occurrence of $\rho > 1$, known as 'Heywood cases', and the existence of negative correlations, which do not allow the calculation of ρ. The main causes for the occurrence of Heywood cases include random sampling variations or violation of one or more assumptions of the triads method. In the first case, a validity coefficient above 1 is acceptable. Empirical negative correlations occur when the true correlations are near zero, i.e., the specific factors of the variable predominate over the latent variable. Increasing the sample size and using more accurate reference methods and biomarkers should reduce the likelihood of negative correlations[14]. The validity coefficients vary from 0 to 1.

Individual usual food intakes, resulting from the two 24 h recalls and with incorporation of information from FFQ as a covariate, were estimated using the multiple source method[41]. This method removes the effect of day-to-day variability and random error in both 24 h recalls. This analysis aimed to verify whether the combination of two dietary assessment methods improves correlations between intake of foods and concentration biomarkers. Random intercept mixed models were used to verify associations between mean food intake and concentration biomarkers, food consumption frequency and concentration biomarkers, or mean nutrient intake and concentration biomarkers while controlling for school and centre as random effects and age and sex as fixed effects.

To evaluate nutrient intake assessment, unadjusted Spearman rank correlations were calculated for vitamin B_{12} status (cobalamin and holo-transcobalamin) *v.* vitamin B_{12} intake, folate status (plasma folate and erythrocyte folate) *v.* folate intake, β-carotene status *v.* β-carotene intake, vitamin C status *v.* vitamin C intake, DHA and EPA status *v.* DHA and EPA intake. Pearson's correlation coefficients (after logarithmic transformation) were included as sensitivity analysis.

Participants were classified into tertiles according to their biomarker status for the particular nutrient. The Kruskal–Wallis one-way ANOVA by ranks was used for testing the equality of population medians of intake among these tertiles.

Results were presented for boys and girls separately and were repeated after exclusion of underreporters. Underreporters were defined using the Goldberg cut-offs[42]. BMR was calculated from age- and sex-specific FAO/WHO/UNU equations.

Underreporting was considered when the ratio of energy intake over the estimated BMR was lower than 0·96[43].

Results

Only 390 adolescents (boys, *n* 163) were included for the food intake assessment evaluation, while for the nutrient intake assessment evaluation 697 (boys, *n* 323) adolescents were

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included. Descriptive characteristics, food consumption frequencies, mean food and nutrient intakes and concentration of selected biomarkers in blood of the study sample can be found in Table 1.

Food intake assessment evaluation

All correlations between food consumption frequency and mean food intake, food consumption frequency and concentration biomarker, and mean food intake and concentration biomarker were significantly positive in all adolescents (*n* 390), except for mean vegetable intake *v.* vitamin C status (data not shown). Except for DHA + EPA status *v.* fish intake, highest correlations were found between food consumption frequencies and mean food intakes (*r* 0·51; $P<0·001$ for fruits and *r* 0·29; $P<0·001$ for vegetables). Overall, observed correlations were higher between food consumption frequency and concentration biomarker than between mean food intake and concentration biomarker, especially for DHA + EPA (*r* 0·35; $P<0·001$ *v.* *r* 0·27; $P<0·001$) (data not shown). Important sex differences were observed. All correlations between food consumption frequency and mean food intake, as well as all correlations between mean food intake and concentration biomarker, were substantially higher among girls than boys (Table 2). All associations between food consumption frequency and concentration biomarker were higher among boys than girls, except for associations with vegetable consumption frequency (Table 2). In girls, highest correlations were found between mean fruit intake and frequency of fruit consumption (*r* 0·56; $P<0·001$), mean fish consumption and % DHA + EPA status (*r* 0·36; $P<0·001$) and mean fish consumption and DHA + EPA concentration (*r* 0·34; $P<0·001$). In boys, highest correlations were found between % DHA + EPA status and frequency of fish consumption (*r* 0·42; $P<0·001$) (Table 2).

For boys, highest validity coefficients were found for frequency of fruit consumption (0·88) and for % DHA + EPA biomarker (0·71). While in girls, the highest validity coefficients were found for fruit consumption frequency (0·76), vegetable consumption frequency (0·74), mean fruit intake (0·90) and % DHA + EPA biomarker (0·69) (Table 3). Except for DHA + EPA, the biomarker often had the lowest validity

Table 1. General characteristics, mean daily food and nutrient intakes, food frequency consumption per d and concentration biomarkers in blood of the sample of European adolescents (Healthy Lifestyle in Europe by Nutrition in Adolescence study)

(Mean values, standard deviations, medians and number of participants)

	Boys (*n* 163)				Girls (*n* 227)			
Food intake assessment evaluation (*n* 390)	*n*	Median	Mean	SD	*n*	Median	Mean	SD
Age (years)	163	14·5	14·6	1·3	227	14·4	14·5	1·2
BMI (kg/m^2)	163	20·0	21·1	4·4	227	20·6	21·2	3·5
Food intake (g/d)								
Vegetables*	163	53	91	112	227	63	87	92
Fruits	163	65	112	143	227	93	119	135
Fish (products)	163	0	19	48	227	0	18	46
Food consumption frequency per d								
Vegetables*	163	0·43	0·55	0·39	227	0·79	0·66	0·38
Fruits	163	0·43	0·60	0·39	227	0·79	0·70	0·43
Fish (products)	163	0·14	0·19	0·25	227	0·14	0·19	0·23
Concentration biomarkers								
Vitamin C (mg/l)	161	10·4	10·3	3·3	211	10·8	10·6	3·3
β-Carotene (ng/ml)	146	221·2	279·9	199·5	205	253·6	281·9	163·3
DHA + EPA (%)†	151	3·1	3·3	0·9	215	3·7	3·8	1·2
DHA + EPA (μmol/l)	151	103·7	112·5	36·7	214	135·3	140·7	51·2
Nutrient intake assessment evaluation (*n* 697)	Boys (*n* 323)				Girls (*n* 374)			
Age (years)	323	14·8	14·8	1·3	374	14·7	14·7	1·2
BMI (kg/m^2)	323	20·4	21·2	4·0	374	20·7	21·2	3·5
Nutrient intake per d								
Vitamin B_{12} (μg)	323	5	7	5	374	4	5	10
Folate (μg)	323	200	218	100	374	169	180	85
β-Carotene (μg)	323	1484	2311	2505	374	1303	2228	2566
Vitamin C (μg)	323	77241	97575	76118	374	79715	95812	84269
DHA + EPA (mg)	323	90	282	505	374	67	281	763
Concentration biomarkers								
Vitamin B_{12} (pmol/l)	317	306·0	334·1	133·3	359	349·0	383·1	162·6
Active vitamin B_{12} (pmol/l)	296	59·9	65·5	35·7	356	59·1	66·1	37·8
Plasma folate (nmol/l)	316	15·6	18·1	10·1	359	15·8	18·2	9·6
Erythrocyte folate (nmol/l)	314	740·0	809·3	376·8	354	709·6	761·6	299·7
β-Carotene (ng/ml)	286	217·3	272·7	199·0	328	251·5	273·5	154·5
Vitamin C (mg/l)	316	10·8	10·4	3·2	356	10·9	10·7	3·2
DHA + EPA (%)†	302	3·2	3·4	1·1	355	3·6	3·7	1·1
DHA + EPA (μmol/l)	301	106·6	116·7	47·6	354	132·0	136·4	47·7

* Excluding potatoes.

† Relative amount of DHA + EPA, expressed as the percentage of total fatty acid concentration.

Table 2. Crude Spearman's rank and Pearson correlation coefficient for FFQ *v.* mean food intake from 24 h recalls; FFQ *v.* biomarker and mean food intake from 24 h recalls *v.* biomarker (Healthy Lifestyle in Europe by Nutrition in Adolescence study), for boys and girls

	FFQ *v.* mean food intake‡					FFQ *v.* biomarker					Mean food intake *v.* biomarker				
	n	Spearman	*P*	Pearson	*P*	*n*	Spearman	*P*	Pearson	*P*	*n*	Spearman	*P*	Pearson	*P*
Boys															
Fruit intake *v.* vitamin C	163	0·423	<0·001	0·442	0·004*	161	0·178	0·024	0·208	0·008*	161	0·065	0·414	0·085	0·285*
Vegetable intake *v.* vitamin C	159	0·240	<0·001	0·227	0·004*	157	0·064	0·427	0·035	0·664*	161	0·013	0·872	−0·0046	0·954*
Fruit intake *v.* β-carotene	163	0·423	<0·001	0·442	<0·001*	146	0·213	0·010	0·250	0·002†	146	0·128	0·124	0·142	0·087†
Vegetable intake *v.* β-carotene	159	0·240	<0·001	0·227	0·004*	142	0·071	0·401	0·086	0·307†	146	0·058	0·487	0·104	0·212†
Fish intake *v.* DHA + EPA (%)§	163	0·195	0·013	0·126	0·110*	151	0·421	<0·001	0·412	<0·001*	151	0·191	0·019	0·153	0·061*
Fish intake *v.* DHA + EPA	163	0·195	0·013	0·126	0·110*	151	0·296	<0·001	0·296	<0·001*	151	0·080	0·331	0·095	0·247*
Girls															
Fruit intake *v.* vitamin C	227	0·564	<0·001	0·523	<0·001*	211	0·146	0·034	0·147	0·033*	211	0·224	0·001	0·226	<0·001*
Vegetable intake *v.* vitamin C	225	0·314	<0·001	0·321	<0·001*	210	0·197	0·004	0·190	0·006*	211	0·096	0·166	0·112	0·104*
Fruit intake *v.* β-carotene	227	0·564	<0·001	0·523	<0·001*	205	0·196	0·005	0·217	0·002†	205	0·186	0·008	0·196	0·005†
Vegetable intake *v.* β-carotene	225	0·314	<0·001	0·321	<0·001*	204	0·293	<0·001	0·257	<0·001†	205	0·280	<0·001	0·290	<0·001†
Fish intake *v.* DHA + EPA (%)§	227	0·244	<0·001	0·213	0·001*	215	0·306	<0·001	0·302	<0·001*	215	0·359	<0·001	0·331	<0·001*
Fish intake *v.* DHA + EPA	227	0·244	<0·001	0·213	0·001*	214	0·281	<0·001	0·292	<0·001*	214	0·341	<0·001	0·297	<0·001*

* After square root transformation of both FFQ and mean food intake, FFQ and nutrient status and mean food intake and nutrient status.
† After square root transformation of food intake and FFQ and log-transformation of nutrient status.
‡ In this column, the correlations reported refer to associations between food consumption frequency and food intake (e.g. consumption frequency of fruits *v.* mean fruit intake).
§ Relative amount of DHA + EPA, expressed as the percentage of total fatty acid concentration.

Table 3. Validity coefficients (triads method) for FFQ, mean food intake from 24 h recalls and biomarker status for both boys and girls

	Boys			Girls		
Triad	ρ_{qi}*	ρ_{ri}†	ρ_{bi}‡	ρ_{qi}*	ρ_{ri}†	ρ_{bi}‡
Fruit intake *v.* vitamin C	1·04	0·43	0·20	0·58	0·90	0·25
Vegetable intake *v.* vitamin C	−§	−§	−§	0·74	0·43	0·26
Fruit intake *v.* β-carotene	0·88	0·50	0·28	0·76	0·69	0·29
Vegetable intake *v.* β-carotene	0·43	0·52	0·20	0·53	0·60	0·48
Fish intake *v.* DHA + EPA (%)‖	0·58	0·22	0·71	0·44	0·48	0·69
Fish intake *v.* DHA + EPA	0·63	0·20	0·47	0·46	0·47	0·64

* Validity coefficient of FFQ.
† Validity coefficient of 24 h recall.
‡ Validity coefficient of concentration biomarker.
§ Not possible to calculate due to negative correlation coefficient.
‖ Relative amount of DHA + EPA, expressed as the percentage of total fatty acid concentration.

coefficient compared to FFQ and 24 h recall. One Heywood case occurred (for boys; for fruit consumption frequency in relation to true intake), where the estimated validity coefficient was >1. The validity of the 24 h recall was the highest in girls, while in boys the validity of the FFQ was higher compared to the 24 h recall.

After exclusion of underreporters (*n* 86), except for the correlation between mean fish consumption and frequency of fish consumption, all correlations slightly improved. For example, *r* 0·51 became *r* 0·54 ($P<0·001$) between mean fruit intake and frequency of fruit consumption and *r* 0·29 became *r* 0·37 ($P<0·001$) between mean vegetable intake and frequency of vegetable consumption (data not shown).

After correction for centre and school as random effects and sex and age as fixed effects, all associations between food consumption frequency and concentration biomarker and between mean food intake and concentration biomarker, were significantly positive (data not shown).

Most correlations between mean usual food intakes, adjusted for food consumption frequency (Table 4), and concentration biomarkers were higher than correlations between mean food intakes and concentration biomarkers (Table 2), especially for fish intake *v.* DHA + EPA status, fruit intake *v.* vitamin C status and fruit intake *v.* β-carotene status. Most of the differences were not significant, but the general trend is remarkable even when some correlations were lower. When examining Spearman rank correlations between usual food intakes and biomarkers (without taking into account food frequency), most of the correlations were equal or slightly higher than when using the mean intakes, but lower than when taking into account food frequency of consumption, which indicates the importance of the FFQ information. The latter was only not the case for fish consumption *v.* DHA + EPA. Usual fish intake correlated better with DHA + EPA than mean fish intake, but when usual fish intake was corrected for frequency of fish consumption, lower correlations were obtained (data not shown).

Nutrient intake assessment evaluation

Overall correlations between mean nutrient intake and concentration biomarkers were low. The highest correlations were found for β-carotene ($\rho = 0·19$ or *r* 0·23; $P<0·001$) and % DHA + EPA ($\rho = 0·16$ or *r* 0·17; $P<0·001$) (data not shown). There were important sex differences. Correlations were higher among girls than boys, except for EPA + DHA (Table 5). For girls highest correlations were found for folate, active vitamin B_{12} and β-carotene, while for boys highest correlations were found for DHA + EPA. For girls, correlations were substantially higher when active vitamin B_{12} was used instead of vitamin B_{12} as a biomarker. Correlations for folate were better when plasma folate instead of erythrocyte folate was used as a biomarker, for both boys and girls. In most cases, correlations slightly or substantially improved after exclusion of underreporters (*n* 142): *r* 0·16 → *r* 0·20; $P<0·001$ for folic acid, *r* 0·14 → *r* 0·20; $P<0·001$ for vitamin C and *r* 0·16 → *r* 0·19; $P<0·001$ for DHA + EPA (data not shown). Spearman rank correlations comparing usual nutrient intakes *v.* biomarkers were equal or slightly higher than spearman rank correlations comparing mean nutrient intakes *v.* biomarkers (data not shown).

After correction for centre and school as random effects and sex and age as fixed effects, all associations between nutrient intake and concentration biomarkers were significantly positive (data not shown). For all nutrients, biomarker status significantly increased with increasing tertile of nutrient intake (Table 6).

Discussion

Except for fish intake *v.* DHA + EPA, highest correlations were found between food consumption frequencies and mean food intakes derived from two independent 24 h recalls. In many cases, correlations were better between food consumption frequency and concentration biomarker than between mean food intake and concentration biomarker, especially for DHA + EPA. This is most probably due to the fact that food consumption frequency represents usual intake while two non-consecutive 24 h recalls represent actual intake, in particular for foods that are generally not consumed daily (e.g. fish). In addition, FA biomarkers in serum phospholipids mirror the dietary intake for recent weeks, while plasma vitamin C and β-carotene reflect short-term dietary intake. It was found previously that dietary intakes

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Table 4. Crude Spearman's rank and Pearson correlation coefficient for usual food intake (corrected for frequency of consumption) *v.* biomarker (Healthy Lifestyle in Europe by Nutrition in Adolescence study)

	All population				Boys				Girls			
	n	Spearman	*P*	Pearson	*P*	*n*	Spearman	*P*	Pearson	*P*	*n*	Spearman	*P*	Pearson	*P*
Fruit intake *v.* vitamin C	372	0·174	<0·001	0·185	<0·001*	161	0·088	0·267	0·127	0·109*	211	0·234	<0·001	0·233	<0·001*
Vegetable intake *v.* vitamin C	367	0·058	0·269	0·066	0·206*	157	-0·023	0·774	0·0001	0·999*	210	0·120	0·084	0·123	0·075*
Fruit intake *v.* β-carotene	346	0·188	<0·001	0·216	<0·001†	142	0·031	0·717	0·102	0·229†	204	0·305	<0·001	0·310	<0·001†
Vegetable intake *v.* β-carotene	351	0·191	<0·001	0·202	<0·001†	146	0·156	0·060	0·166	0·046†	205	0·207	0·003	0·230	<0·001†
Fish intake *v.* DHA + EPA (%)‡	366	0·289	<0·001	0·315	<0·001*	151	0·245	<0·001	0·236	0·004*	215	0·352	<0·001	0·377	<0·001*
Fish intake *v.* DHA + EPA	365	0·221	<0·001	0·257	<0·001*	151	0·123	0·133	0·164	0·044*	214	0·310	<0·001	0·330	<0·001*

* After square root transformation of both usual nutrient intake and nutrient status.
† After square root transformation of usual nutrient intake and log-transformation of nutrient status.
‡ Relative amount of DHA + EPA, expressed as the percentage of total fatty acid concentration.

correlate better with biomarkers when the number of days covered by the reference method increases[44]. Most correlations between mean usual food intakes, taking into account food consumption frequency, and concentration biomarkers were higher than correlations between mean food intakes and concentration biomarkers, especially for DHA + EPA *v.* fish intake, vitamin C *v.* fruit intake and β-carotene *v.* fruit intake. Consequently, it can be recommended to combine information from different dietary assessment methods to estimate dietary intake. Compared to the European Food Consumption Validation study among adults[7], correlations between usual fish intake (assessed combining 24 h recall and FFQ) and DHA + EPA were similar though slightly higher in our study.

Except for DHA + EPA, biomarkers often had the lowest validity coefficient compared to FFQ and 24 h recall. The fact that biomarkers do not always perform better than other food intake assessment methods was observed previously[45]. Moreover, not all nutrients have biological markers and many are influenced by other factors than intake. The weak correlations result from factors related to absorption, post-absorptive metabolism or physiological regulation of nutrient levels, which can be important sources of random variations in the markers, unrelated to true intake[10,46]. The quantitative relationship of biomarkers with intake may differ among individuals; they cannot be transformed into absolute estimates of ingestion[47]. Hence, biomarkers should be used in addition to and not in replacement of dietary surveys.

The fact that biomarkers had the lowest validity coefficients could be due to the fact that there was a positive co-variance between the random errors of the questionnaire and 24 h recalls and thus that validity coefficients in relation to true intake of FFQ and 24 h recalls have been overestimated and should be interpreted as upper limits. In the latter case, the validity coefficients of biomarkers in relation to true intake could be underestimated[14]. Overall correlations between mean nutrient intake and concentration biomarkers were low. The highest correlations were found for β-carotene and % DHA + EPA.

The results showed important sex differences. All correlations between food consumption frequency and mean food intake, as well as all correlations between mean food intake and concentration biomarkers, were substantially higher among girls than boys. All associations between food consumption frequency and concentration biomarkers, except for associations with vegetable consumption frequency, were higher among boys than girls. For girls, the highest correlations were found between mean fruit intake and frequency of fruit consumption, mean fish consumption and % DHA + EPA status, and mean fish consumption and DHA + EPA concentration. For boys, the highest correlations were found between % DHA + EPA status and frequency of fish consumption. Except for DHA + EPA, correlations were better among girls than boys. For both, the evaluation of food and nutrient intake assessment correlations improved slightly after exclusion of underreporters, which is an interesting finding and could plead for the exclusion of underreporters when investigating diet–disease relationships.

Table 5. Crude Spearman's rank and Pearson correlation coefficient for mean nutrient intake (dietary recall) *v.* nutrient status (biomarkers in blood samples) (Healthy Lifestyle in Europe by Nutrition in Adolescence study), for boys and girls separately (*n* 697)

	Mean nutrient intake (dietary recall) *v.* nutrient status									
	Boys (*n* 323)					Girls (*n* 374)				
Nutrient	*n*	Spearman	*P*	Pearson*	*P*	*n*	Spearman	*P*	Pearson*	*P*
Vitamin B_{12}	317	0·164	0·004	0·143	0·011	359	0·149	0·005	0·154	0·003
Vitamin B_{12} (active)	296	0·157	0·007	0·140	0·016	356	0·198	<0·001	0·166	0·002
Folate	316	0·124	0·027	0·110	0·062	359	0·216	<0·001	0·207	<0·001
Folate (erythrocyte)	314	0·073	0·199	0·033	0·556	354	0·166	0·002	0·160	0·003
β-Carotene	286	0·135	0·022	0·164	0·005	328	0·261	<0·001	0·284	<0·001
Vitamin C	316	0·095	0·093	0·124	0·028	356	0·172	0·001	0·156	0·003
DHA + EPA (proc)	302	0·203	<0·001	0·172	0·003	355	0·152	0·004	0·177	<0·001
DHA + EPA (conc)	301	0·176	0·002	0·179	0·002	354	0·137	0·010	0·152	0·004

* After log-transformation.

The HELENA study has several strengths. The sampling procedure and the strict standardisation of the fieldwork among the countries involved in the study avoided introduction of bias due to inconsistent protocols and different laboratory methods, which in turn makes comparing results from isolated studies difficult. The combination of repeated 24 h recalls, FFQ and biomarker information allowed an in-depth investigation of the validity of the dietary intake assessment methods used in the HELENA study. Furthermore, the sample size was large enough for validation studies with biomarkers as the reference method[48].

This study is not without limitations. Data are observational, based on self-reported dietary intake and are mean values of two non-consecutive 24 h recalls, which is probably not enough time to accurately capture usual intake, especially for children and adolescents where the ratio of within-to between-subject variability is larger[49]. It is important to remember that current blood concentrations of vitamins in the adolescent population do not necessarily mean that these concentrations are the most adequate ones from the biological point of view. Furthermore, no detailed information on supplement intake was available from the HELENA study and this might have affected the observed correlations between the biomarkers and food intake assessment. Finally, the study sample is not nationally representative and thus the results may not be generalised to broader adolescent populations, in the countries surveyed or elsewhere. Furthermore, for the food intake assessment evaluation, many adolescents were lost in subsequent analysis because they did not complete one or more of the questions in the FFQ. Therefore, the sample size for the food validations was much lower than for the nutrient validations, since this latter validation did not include the FFQ data. Further nutrient intake estimations were based on the German food composition table and may have introduced some bias in the nutrient intake calculations. However, preliminary analyses comparing the differences in nutrient intake estimates of eight nutrients when using the BLS table for all HELENA countries in comparison with the approach in which all countries used their national food composition table to calculate the nutrient intakes have shown that differences between these two approaches are only small and for most nutrients negligible (I Huybrechts, unpublished results). It is, however, clear from this study that correlations between biomarkers and food intake assessment (which does not use food composition tables) are better than between biomarkers and nutrient intake assessment.

Conclusion

The present study shows that two non-consecutive 24 h recalls in combination with a FFQ seem to be appropriate to rank subjects according to their usual food intake.

Table 6. Average nutrient status (biomarker) by tertiles of nutrient intake (24 h recall) (*n* 697)
(Mean values, standard deviations and number of participants)

	Tertile 1 nutrient intake			Tertile 2 nutrient intake			Tertile 3 nutrient intake			
Nutrient status	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*P**
Vitamin B_{12} (pmol/l)	218	336·2	137·4	228	364·0	155·3	230	379·0	157·9	0·012
Active B_{12} (pmol/l)	211	58·3	27·7	220	67·7	38·0	221	71·1	41·9	<0·001
Plasma folate (nmol/l)	222	16·5	8·8	225	18·1	9·9	228	19·9	10·5	<0·001
Erythrocyte folate (nmol/l)	218	744·5	349·1	222	767·5	298·9	228	837·9	359·2	0·005
β-Carotene (ng/ml)	211	240·1	139·2	205	275·3	186·6	198	306·2	194·9	<0·001
Vitamin C (mg/l)	220	10·2	3·3	222	10·4	3·1	230	11·2	3·1	0·003
DHA + EPA (%)†	217	3·4	1·0	213	3·4	1·0	227	3·9	1·3	<0·001
DHA + EPA (μmol/l)	215	123·6	43·4	213	118·9	41·7	227	138·8	56·8	0·001

* *P* from Kruskal–Wallis.
† Relative amount of DHA+EPA, expressed as the percentage of total fatty acid concentration.

Based on the results of this study, the use of a combination of dietary assessment methods such as 24 h recall and FFQ is recommended to estimate dietary intake, especially for foods which are not consumed on a daily basis such as fish. Besides, the exclusion of underreporters might be beneficial.

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